Oestrogen deficiency impairs intestinal calcium absorption in the rat

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1. The effects of ovariectomy on the relationships between calcium consumption and calcium balance and its components were assessed in adult (10–14 months) sham-operated and ovariectomized (Ovx) rats fed a semi-synthetic diet with the calcium content varying between 0·02 and 0·4 %.

2. Adaptation to dietary calcium restriction was monitored for 47 days from commencement of a 0·02 % Ca diet.

3. Response to 1,25-dihydroxyvitamin D (20 ng kg^{-1} day^{-1}) administration in sham and Ovx rats and oestradiol (E2) (20 μg kg^{-1} day^{-1}) replacement in Ovx rats was assessed in rats fed a 0·05 % Ca diet.

4. Ovx rats had lower intercepts for the relationships between calcium consumption and both calcium balance ($P < 0·005$) and intestinal calcium absorption ($P < 0·005$) compared with sham rats, but 1,25-dihydroxyvitamin D was not reduced in Ovx rats.

5. The magnitude of adaptation to dietary calcium restriction was unaffected by ovariectomy.

6. Intestinal calcium absorption was stimulated by an equivalent amount in sham and Ovx rats following 1,25-dihydroxyvitamin D administration, although this did not reach statistical significance for sham (sham, $t = 1·91$, n.s.; Ovx, $t = 3·18$, $P < 0·05$).

7. Oestradiol replacement in Ovx rats induced a marked increase in intestinal calcium absorption ($t = 8·25$, $P < 0·005$), without stimulating circulating 1,25-dihydroxyvitamin D levels and led to a marked increase in calcium balance ($t = 6·89$, $P < 0·005$).

8. These data indicate that the impairment of intestinal calcium absorption following ovariectomy is not the result of reduced circulating 1,25-dihydroxyvitamin D or reduced intestinal responsiveness to 1,25-dihydroxyvitamin D. Moreover E2 stimulates intestinal calcium absorption probably by a direct effect on the intestine.

The mechanism by which ovarian hormones modulate bone mass is not fully understood, but the menopause is associated with increased renal excretion of calcium (Nordin *et al.* 1991) and decreased intestinal calcium absorption (Heaney *et al.* 1989). The reduced calcium absorption has been attributed variously to reduced circulating 1,25-dihydroxyvitamin D levels (Gallagher *et al.* 1979) and to gastrointestinal resistance to the action of 1,25-dihydroxyvitamin D (Gennari *et al.* 1990; Morris *et al.* 1991). In addition, the role of ovarian hormones on adaptation to dietary calcium restriction is unclear, although it has been shown in humans that oestrogens modulate the end organ effect of 1,25-dihydroxyvitamin D on intestinal calcium absorption (Gennari *et al.* 1990).

Ovariectomy in the rat leads to a reduction in bone density at a number of skeletal sites (Wronski *et al.* 1988; Kalu *et al.* 1989; Yamazaki & Yamaguchi, 1989) and in the young rat causes an increase in intestinal calcium secretion, leading to impaired calcium balance (O'Loughlin & Morris, 1994). The effect of ovariectomy on urine calcium excretion is currently controversial with no effect (Morris *et al.* 1992; O'Loughlin & Morris, 1994), an inconsistent effect (Yamazaki & Yamaguchi, 1989) and an increase in urine calcium excretion (Morris *et al.* 1995) having been reported. Net intestinal calcium absorption in rats fed a low calcium diet is impaired by ovariectomy (Kalu *et al.* 1989); however, whether this impairment is the result of a reduction in true calcium absorption or increased intestinal calcium secretion is unclear. A decrease in intestinal 1,25-dihydroxyvitamin D receptor number (Chan *et al.* 1984) following ovariectomy may contribute to reduced intestinal calcium absorption.

The present study reports the relationship between calcium consumption and calcium balance and its components,

including true intestinal calcium absorption and intestinal calcium secretion in both sham-operated controls and ovariectomized adult rats. In addition, the effect of ovariectomy on adaptation to dietary calcium restriction and the response to treatment with 1,25-dihydroxy-vitamin D and oestradiol replacement in ovariectomized rats are presented.

METHODS

The modified AIN-76A-starch (American Institute of Nutrition, 1977) semi-synthetic diet (Table 1) was prepared from the following materials; casein (Bonlac Foods, Melbourne, Victoria, Australia), cellulose (James River Corporation, Berlin, NH, USA), cornstarch (Goodman Fielder Mills, Summer Hill, NSW, Australia), DL-methionine, choline bitartrate (Sigma Chemical Co.), AIN Vitamin Mix (ICN, Costa Mesa, CA, USA) and corn oil (Lion and Globe, Singapore). The calcium content of the diet was varied within the range 0·02–0·4% by the addition of calcium carbonate (BDH Laboratory Supplies, Poole, Dorset, UK) to an otherwise calcium-free diet. All diets contained 0·3% P and 4000 i.u. vitamin D (kg diet)$^{-1}$.

Calcium balance measurement

Calcium balance was measured by a modification of a method previously published (O'Loughlin & Morris, 1994). Rats were placed in individual metabolic cages (Techniplast, Buguggiate, Italy) for a 4 day adaptation period, with 2 MBq of ^{45}Ca ($CaCl_2$, 83·6 GBq l^{-1}: Amersham) administered intramuscularly on day 4 to monitor the secretion of endogenous calcium into the gut. During the adaptation period, food was freely available and consumption was monitored. From day 5 to the completion of the balance study, rats were fed 90% of the mean food consumption of the ovary-intact animals during the adaptation period. Calcium balance was determined over a 6 day period (days 6–11). Urine samples were collected into 10 N HCl. Any food remaining at the end of each 24 h period was collected, weighed and the exact consumption recorded. On completion of the 6 day calcium balance period, urine and faeces samples were collected and weighed.

Triplicate 10 g samples of the diet and entire 6 day faecal samples were charred in porcelain crucibles in a muffle furnace (Tetlow, Melbourne, Victoria, Australia) by raising the temperature gradually to 400 °C over 4 h and then ashed at 800 °C for 18 h. The ash was dissolved in 5 ml of 5 N HCl, warmed to approximately 75 °C for 5 min and made up to exactly 10 ml with 5 N HCl. The pH of urine samples was adjusted to <2 with HCl. Faecal ^{45}Ca and urine ^{45}Ca were determined by liquid scintillation counting (Packard Minaxi, Tri-carb 4000, Downers Grove, IL, USA). Faecal ^{40}Ca, urine ^{40}Ca and diet ^{40}Ca were determined by atomic absorption spectrometry (model 3030, Perkin-Elmer, Norwalk, CT, USA).

Calculations. Calculation of calcium balance, endogenous faecal calcium, intestinal calcium secretion and calcium absorption were calculated by the following equations (Nordin *et al.* 1976):

Ca consumption (mmol day^{-1}) = mean food consumption × food ^{40}Ca.

Ca balance (mmol day^{-1}) = Ca consumption − (faecal ^{40}Ca + urine ^{40}Ca).

Endogenous faecal Ca (mmol day^{-1}) = urine ^{40}Ca × (faecal ^{45}Ca/urine ^{45}Ca).

Unabsorbed dietary calcium (mmol day^{-1}) = faecal ^{40}Ca − endogenous faecal Ca.

Fractional calcium absorption (mmol day^{-1}) = (Ca consumption − unabsorbed dietary Ca)/Ca consumption.

True Ca absorption = fractional calcium absorption × 100.

Intestinal Ca secretion (mmol day^{-1}) = endogenous faecal Ca/(1 − fractional Ca absorption).

Intestinal calcium secretion was estimated in rats fed between 0·05 and 0·4% dietary calcium.

Hormone analyses

1,25-Dihydroxyvitamin D was determined by radioimmunoassay following Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) and high-performance liquid chromatography (Taylor *et al.* 1980). Oestradiol was measured in unextracted serum samples by a chemiluminescence immunoassay (Ciba-Corning, 1994) on an ACS:180 immunoassay analyser (Ciba-Corning Diagnostics Corporation, Medfield, MA, USA). Parathyroid hormone (PTH) was determined by a two-site immunoradiometric assay specific for rat PTH (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

Experimental protocol

Female Sprague–Dawley rats were purchased from Gilles Plains Animal Research Centre (Gilles Plains, SA, Australia) and randomly allocated at 7 months of age to either ovariectomy (Ovx) or sham operation, via a single ventral incision under halothane anaesthesia (2% in O_2:N_2O (2:1)) delivered by face mask. Calcium balance studies were conducted when animals were between 10 and 14 months of age. The experimental protocol was approved by the institutional animal ethics committee.

Experiment 1. Adaptation to dietary calcium restriction and the effect of ovariectomy. Adaptation to dietary calcium restriction was assessed in five sham-operated and five Ovx rats. The animals were maintained on a 0·4% calcium diet from 2 weeks prior to operation until 15 weeks after operation (11·5 months of age), at which time they began a 0·02% calcium diet. The ability to adapt to the calcium-restricted diet was assessed by calcium balance studies performed at 5, 26 and 47 days after commencement of the 0·02% calcium diet. In experiments 2 and 3, a minimum period of adaptation of 8 weeks before measurement of calcium balance was adopted on the basis of the data obtained from experiment 1.

Experiment 2. The effect of ovariectomy on calcium balance and calcium absorption at varying levels of dietary calcium. After operation, forty female Sprague–Dawley rats were separated into groups of five sham and five Ovx and placed on one of the following dietary calcium levels: 0·02, 0·05, 0·1, 0·15, 0·2 or 0·4%. The rats were maintained on the allocated diet for at least 10 weeks before measurement of calcium balance and its components. On completion of a balance measurement, the rats were reallocated to another dietary calcium level for another period of at least 10 weeks and the balance measured again, until the calcium balance measurement was performed on at least five rats in each group at each dietary calcium level.

Experiment 3. The effect of 1,25-dihydroxyvitamin D administration and oestradiol replacement on intestinal calcium absorption and calcium balance. Twenty female Sprague–Dawley rats were randomly allocated to either ovariectomy or sham operation at 7 months of age. The rats were fed the 0·05% calcium diet from 8 weeks before the operation until the end of the experiment. At 11 weeks post operation calcium balance was assessed in all animals, following which ten of the animals (5 sham

Table 1. Composition of AIN-76A*-starch semi-synthetic diet (0·02–0·4% calcium)

Ingredient	Amount (g (100 g diet)$^{-1}$)
Casein	20
Corn starch	65
Cellulose	5
Corn oil	5
DL-Methionine	0·3
Choline bitartrate	0·2
Calcium-depleted AIN 76 mineral mix†	3·5
AIN 76A vitamin mix‡	1
Calcium carbonate (0·02–0·4%)	0·5–10

* American Institute of Nutrition (1977). † Composition of calcium-depleted AIN-76 mineral mix was as follows (mg (kg diet)$^{-1}$): sodium dihydrogen phosphate ($2H_2O$), 6895; potassium dihydrogen phosphate, 9625; potassium sulphate, 1820; magnesium oxide, 840; manganous carbonate, 123; ferric citrate, 210; zinc carbonate, 56; cupric carbonate, 11; potassium iodate, 0·4; sodium selenite, 0·4; chromium potassium sulphate, 19; finely powdered sucrose, 15400. ‡ Composition of AIN-76A vitamin mix (ICN, Costa Mesa, CA, USA) was as follows (mg (kg diet)$^{-1}$): thiamine hydrochloride, 6; riboflavin, 6; pyridoxine hydrochloride, 7; nicotinic acid, 30; D-calcium pantothenate, 16; folic acid, 2; D-biotin, 0·2; cyanocobalamin, 0·01; retinyl palmitate, 16; DL-α-tocopherol acetate, 200; cholecalciferol, 2·5; menadione sodium bisulphite complex, 0·5; finely powdered sucrose, 9729.

and 5 Ovx) were killed under halothane anaesthesia by cervical dislocation. Blood was collected from the tail vein and by cardiac puncture for parathyroid hormone and 1,25-dihydroxyvitamin D analyses, respectively.

1,25-Dihydroxyvitamin D treatment. 1,25-Dihydroxyvitamin D was administered at 20 ng kg^{-1} day^{-1} to the remaining five sham and five Ovx rats by a subcutaneous 14 day mini-osmotic pump (Alzet 2002, Alza Corporation, Palo Alto, CA, USA) from 8 days prior to the commencement of the calcium balance measurement period (14 weeks post operation) until the end of the balance measurement period. On completion of the balance measurement, blood was collected from the tail vein for parathyroid hormone and 1,25-dihydroxyvitamin D analyses.

Oestradiol replacement in ovariectomized rats. At 19 weeks post operation a further assessment of calcium balance was performed before 17β-oestradiol (20 μg kg^{-1} day^{-1}) was administered via subcutaneous 14 day mini-osmotic pumps inserted into ovariectomized rats at 13 days and 1 day prior to commencement of the period of balance measurement. The balance after oestradiol replacement was measured at 23 weeks post operation. Immediately following the balance measurement the rats were killed under halothane anaesthesia by cervical dislocation. Blood was collected from the tail vein for parathyroid hormone analysis and by cardiac puncture for 1,25-dihydroxyvitamin D and oestradiol analyses, respectively.

Statistical analyses

The data are expressed as means ± S.E.M. The effect of ovariectomy and time were analysed by two-way ANOVA. To determine the function that best described the relationship between calcium consumption and faecal calcium, the consumption values were standardized for each mathematical function by subtracting the mean from fitted values and a diagnostic plot of residuals was used to eliminate the functions with systematic lack-of-fit (Draper & Smith, 1966). A similar method was used to determine the relationships between calcium consumption and calcium balance and its components. Student's two-tailed t test was used to assess differences between operation groups and Student's two-tailed paired t test was used to assess the effects of treatment with 1,25-dihydroxyvitamin D or oestradiol. Differences were considered significant at $P < 0·05$.

RESULTS

Experiment 1. Adaptation to dietary calcium restriction

There was a significant increase in calcium balance in both sham and Ovx groups between 5 and 47 days after commencement of dietary calcium restriction (Fig. 1*A*) ($P < 0·0001$), although that of the Ovx group was significantly lower than that of the sham group at each time point throughout this period ($P < 0·02$). Net calcium absorption also increased significantly in both groups between 5 and 47 days following commencement of dietary calcium restriction (Fig. 1*B*) ($P < 0·0001$) but, as for calcium balance, the net calcium absorption of the Ovx group remained lower than that for the sham group for each time point ($P < 0·02$). Urine calcium excretion decreased in the sham group from day 5 ($(2·3 \pm 0·66) \times 10^{-3}$ mmol day^{-1}) to day 47 ($(0·85 \pm 0·24) \times 10^{-3}$ mmol day^{-1}) ($P < 0·001$) and in the Ovx group from day 5 ($(2·9 \pm 0·24) \times 10^{-3}$ mmol day^{-1}) to day 47 ($(1·25 \pm 0·25) \times 10^{-3}$ mmol day^{-1}) ($P < 0·001$), but there was no difference between the two operation groups.

Serum 1,25-dihydroxyvitamin D levels after 12 weeks on a 0·02% Ca diet were significantly higher in the Ovx group compared with the sham group and in both groups levels were higher than in data obtained for rats fed the same modified AIN-76A-starch diet containing 0·4% Ca (Mason & Morris, 1997) (Table 2). PTH levels after 12 weeks on the 0·02% Ca diet were not affected by ovariectomy, but were higher than those obtained for rats fed 0·4% calcium (Table 2).

Experiment 2. Relationship between calcium consumption and the components of calcium balance

The relationship between calcium consumption and faecal calcium was best described by a quadratic polynomial function. The relationships between standardized consumption and faecal calcium for both the sham and ovariectomized groups were highly statistically significant (sham $r^2 = 0·99$, Ovx $r^2 = 0·99$). When these relationships were compared by general linear modelling, the difference in the intercepts was statistically significant (intercepts: sham, $+0·48 \pm 0·013$; Ovx, $+0·53 \pm 0·015$; $F = 4·6$; $P < 0·05$), but there was no difference between the slopes or the quadratic terms for the two groups. From the relationships for the two operational groups for dietary calcium levels

between 0·02 and 0·2% the intercept for the relationship in the ovariectomized rats was 20% higher than for the sham-operated rats and highly significant (intercepts: sham, $+0{\cdot}25 \pm 0{\cdot}009$; Ovx, $+0{\cdot}3 \pm 0{\cdot}009$); $F = 12{\cdot}8$; $P < 0{\cdot}001$). There was no effect of ovariectomy on either the slope or the quadratic term.

There was a logarithmic relationship between calcium balance and calcium consumption in both the sham and ovariectomized rats (Fig. 2*A*). General linear modelling analysis indicated that the intercept for the Ovx group was significantly lower than that for the sham group ($P < 0{\cdot}005$). There was no statistically significant difference between the slopes of these equations for the two operation groups. The relationship between net intestinal calcium absorption and calcium consumption was also described by a logarithmic function in both the sham and ovariectomized rats (Fig. 2*B*) with the intercept for the Ovx group again significantly lower than that for the sham group ($P < 0{\cdot}005$). There was no statistically significant difference between the slopes of these equations for the two operation groups.

The relationship between intestinal calcium secretion and calcium consumption was best described by a logarithmic

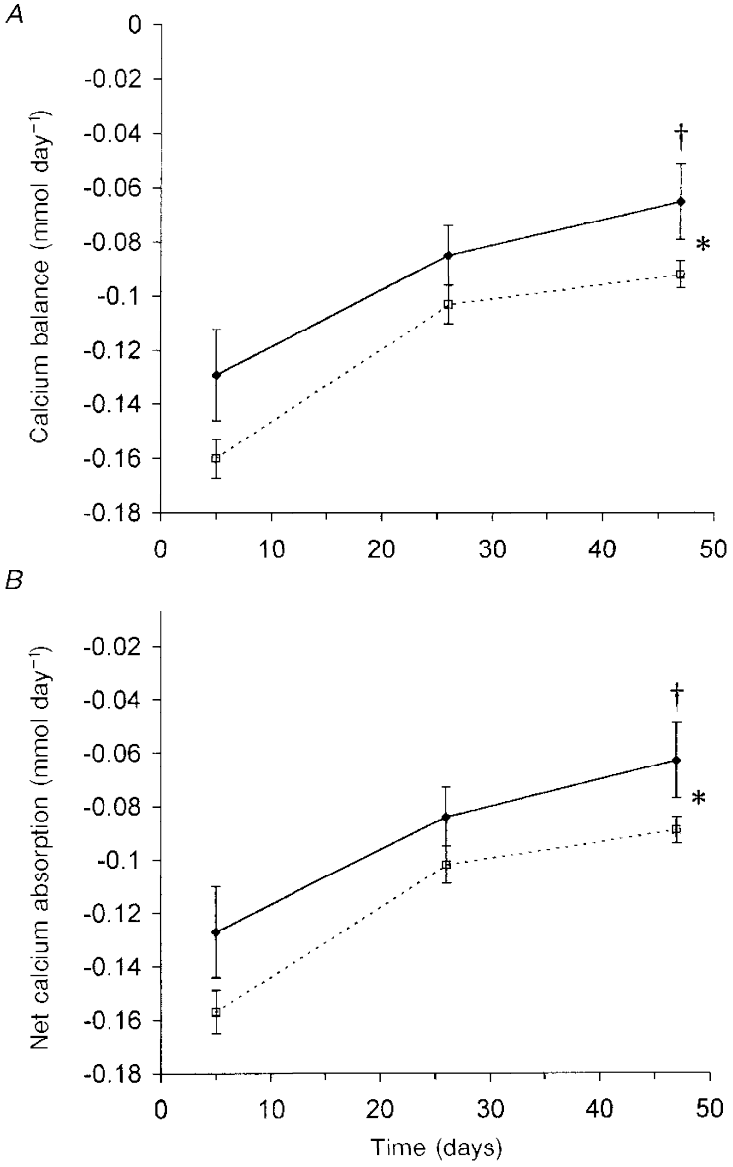

Figure 1. Adaptation to dietary calcium restriction

A, adaptation in calcium balance to dietary calcium restriction in sham-operated control (◆) and ovariectomized (□) rats for 47 days after commencement of 0·02% Ca diet. *B*, adaptation in net intestinal calcium absorption to dietary calcium restriction in sham-operated control (◆) and ovariectomized (□) rats for 47 days after commencement of 0·02% Ca diet. The data are expressed as means ± S.E.M. for 5 rats. * $P < 0{\cdot}0001$, effect of time after commencement of dietary calcium restriction; † $P < 0{\cdot}02$, effect of Ovx compared with sham group.

function and was unaffected by ovariectomy (intestinal Ca secretion = 0·039 + 0·024 × log(Ca consumption), r^2 = 0·65). The relationship between urinary excretion of calcium and calcium consumption was also best described by a logarithmic function and was unaffected by ovariectomy (urine Ca excretion = 0·008 + 0·004 × log(Ca consumption), r^2 = 0·27).

Experiment 3. Hormone treatment

1,25-Dihydroxyvitamin D administration. 1,25-dihydroxyvitamin D significantly increased circulating 1,25-dihydroxyvitamin D levels in sham and Ovx rats fed 0·05% dietary calcium, but there was no change in PTH levels (Table 2). Fractional absorbed calcium increased by an

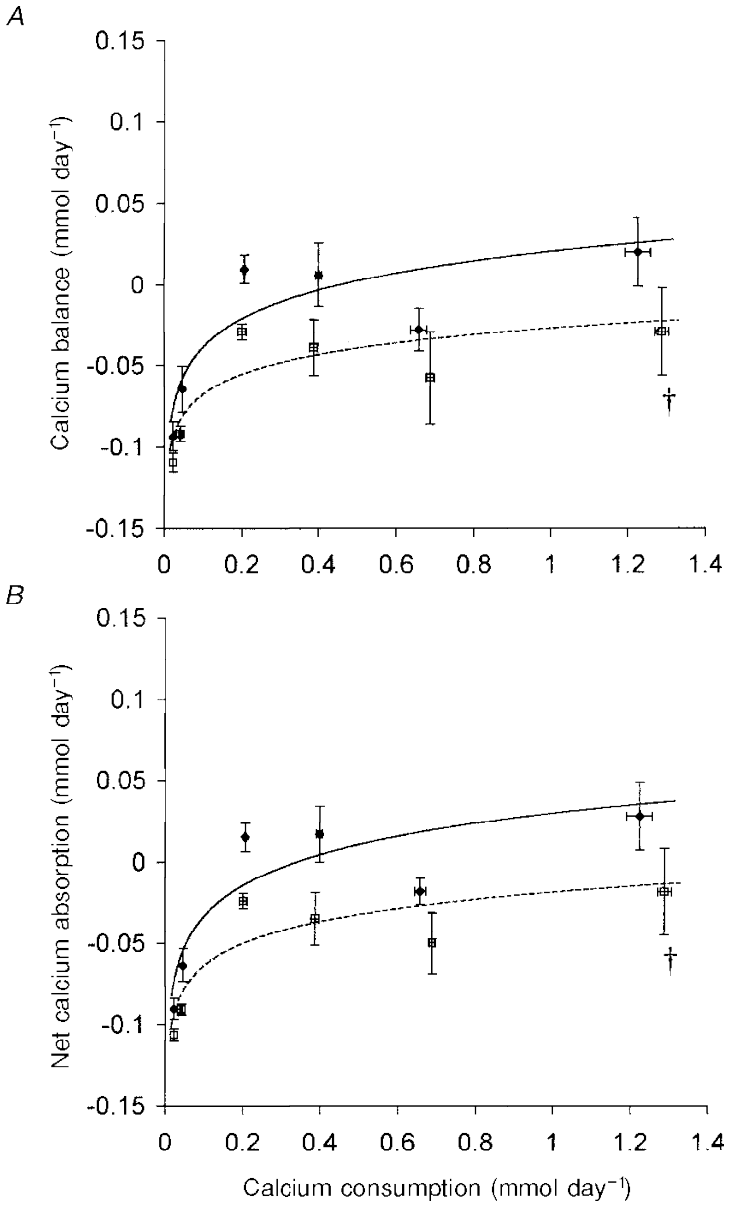

Figure 2. The effect of dietary calcium on calcium absorption and calcium balance

A, the relationship between calcium consumption and calcium balance for sham-operated control (◆) and ovariectomized (□) rats fed diets containing between 0·02 and 0·4% Ca. The relationships were described by the following equations. Sham: calcium balance = + 0·0204 + 0·059 × log(calcium consumption), r^2 = 0·319; Ovx: calcium balance = −0·0269 + 0·0407 × log(calcium consumption), r^2 = 0·181. *B*, the relationship between calcium consumption and net intestinal calcium absorption for sham-operated control (◆) and ovariectomized (□) rats fed diets containing between 0·02 and 0·4% Ca. The relationships were described by the following equations. Sham: net calcium absorption = + 0·03 + 0·0633 × log(calcium consumption), r^2 = 0·371; Ovx: net calcium absorption = −0·0184 + 0·0453 × log(calcium consumption), r^2 = 0·219. The data are expressed as means ± S.E.M. for 5–10 rats. † $P < 0·005$, effect of Ovx on intercept for the relationship.

Table 2. Serum 1,25-dihydroxyvitamin D and parathyroid hormone levels before and after dietary calcium restriction or hormone treatment

	Dietary Ca level (%)	1,25-Dihydroxyvitamin D (pmol l^{-1})		PTH (pmol l^{-1})	
		Sham	Ovx	Sham	Ovx
Before dietary Ca restriction [a]	0·4	83 ± 19·6	104 ± 22·9	6·2 ± 0·2	3·4 ± 0·6‡‡
After 12 weeks on 0·02% Ca	0·02	140 ± 7·1*	198 ± 25·6*‡	13·9 ± 1·8**	12·1 ± 2·6**
Before hormone treatment	0·05	150 ± 9·2	156 ± 7·0	13·0 ± 1·2	11·5 ± 1·7
Post 1,25-dihydroxyvitamin D	0·05	229 ± 22†	211 ± 21†	13·3 ± 1·6	11·7 ± 2·2
Post oestradiol	0·05	—	168 ± 10	—	25·2 ± 2·8††

[a] Data from Mason & Morris (1997): means ± S.E.M. for 4–8 rats. All other values are means ± S.E.M. for 5 rats. * $P < 0·05$, ** $P < 0·01$ compared with 0·4% dietary calcium level; † $P < 0·05$, †† $P < 0·01$ compared with the same dietary calcium level before hormone treatment; ‡ $P < 0·05$, ‡‡ $P < 0·01$ compared with sham for the same dietary calcium level.

Table 3. The response of calcium balance and its components to administered 1,25-dihydroxyvitamin D (1,25D) in sham and ovariectomized rats

	Balance (mmol day^{-1})	Net absorption (mmol day^{-1})	Intestinal calcium secretion (mmol day^{-1})	Fractional calcium absorption (mmol day^{-1})	True calcium absorption (%)	Urine calcium (mmol day^{-1})	Faecal calcium (mmol day^{-1})
Sham							
Baseline	0·025 ± 0·02	0·029 ± 0·026	0·016 ± 0·0005	0·042 ± 0·019	19·1 ± 8·5	0·0038 ± 0·0006	0·191 ± 0·02
+ 1,25D	0·051 ± 0·008	0·06 ± 0·009	0·031 ± 0·002	0·079 ± 0·008	38·6 ± 3·3	0·010 ± 0·002	0·144 ± 0·005
Paired t (P)	1·14 (n.s.)	1·68 (n.s.)	8·1 (0·0013)	1·91 (n.s.)	2·30 (0·083)	3·23 (0·032)	2·49 (0·068)
Ovariectomy							
Baseline	−0·03 ± 0·012	−0·026 ± 0·011	0·02 ± 0·005	−0·005 ± 0·009	−4·4 ± 6·84	0·005 ± 0·001	0·215 ± 0·01
+ 1,25D	−0·003 ± 0·002	0·007 ± 0·003	0·032 ± 0·001	0·03 ± 0·003	18·6 ± 2·5	0·01 ± 0·001	0·18 ± 0·02
Paired t (P)	2·07 (n.s.)	1·93 (n.s.)	2·48 (0·068)	3·18 (0·034)	2·83 (0·047)	5·77 (0·005)	3·93 (0·017)

Values are means ± S.E.M. ($n = 5$). P is the result of Student's paired t test. n.s., not significant.

Table 4. The response of calcium balance and its components to oestradiol (E2) replacement in ovariectomized rats

	Balance (mmol day^{-1})	Net absorption (mmol day^{-1})	Intestinal calcium secretion (mmol day^{-1})	Fractional calcium absorption (mmol day^{-1})	True calcium absorption (%)	Urine calcium (mmol day^{-1})	Faecal calcium (mmol day^{-1})
Baseline	−0·029 ± 0·011	−0·025 ± 0·011	0·015 ± 0·005	−0·01 ± 0·014	−5·0 ± 7·0	0·004 ± 0·001	0·21 ± 0·015
+ E2	0·053 ± 0·01	0·061 ± 0·009	0·031 ± 0·013	0·08 ± 0·008	45·8 ± 4·4	0·008 ± 0·003	0·11 ± 0·01
Paired t (P)	6·89 (0·0023)	13·9 (0·0002)	1·78 (n.s.)	8·25 (0·0012)	13·9 (0·0002)	2·39 (0·075)	12·6 (0·0002)

Values are means ± S.E.M. ($n = 5$). P is the result of Student's paired t test. n.s., not significant.

equivalent amount in the sham and ovariectomized rats when treated with 1,25-dihydroxyvitamin D and the rise achieved statistical significance in the Ovx group (Table 3). There was also an increase in urine calcium excretion in both the sham and ovariectomized groups, a significant rise in intestinal calcium secretion in the sham group and a very strong trend for a rise in intestinal calcium secretion in the ovariectomized group (Table 3). Consequently, the rise in calcium balance following administration of 1,25-dihydroxyvitamin D did not achieve statistical significance in either sham or ovariectomized rats.

Oestradiol administration. Circulating oestradiol levels in the ovariectomized group following oestradiol replacement were significantly increased compared with untreated sham rats (sham, 361 ± 67 pmol l^{-1}; Ovx + E2, 927 ± 211 pmol l^{-1}; $t = 2·56$, $P < 0·05$). Oestradiol replacement in

the ovariectomized group elicited a highly significant rise in true intestinal calcium absorption and calcium balance (Table 4). Increases were also observed for intestinal calcium secretion and urine calcium excretion, but these were not statistically significant. Circulating levels of 1,25-dihydroxyvitamin D were unaffected by oestradiol administration in ovariectomized rats when compared with untreated ovariectomized rats or untreated sham rats on the same diet (Table 2). However, oestradiol stimulated a twofold rise in PTH levels.

DISCUSSION

Both sham and ovariectomized rats exhibited a significant improvement in calcium balance between 5 and 47 days after the commencement of the 0·02% calcium diet with the increase in net intestinal calcium absorption contributing approximately 97% to this adaptation. Urine calcium excretion was reduced by more than 50% with the 0·02% Ca diet but this contributed only about 2% to the adaptation. The ovariectomized rats were unable to achieve the same calcium balance or net absorption as the sham rats at any given time point despite equivalent increments of adaptation in both calcium balance and net absorption to dietary calcium restriction and an equivalent rise in circulating 1,25-dihydroxyvitamin D. This finding suggests that the mechanism of adaptation to dietary calcium restriction is not impaired by ovariectomy. However, the increased levels of 1,25-dihydroxyvitamin D in the ovariectomized rats were unable to stimulate intestinal calcium absorption to the levels occurring in the sham rats and consequently restore calcium balance to that of the controls.

In young rats, dietary calcium restriction increases circulating PTH stimulating renal production and increasing circulating levels of 1,25-dihydroxyvitamin D (Rader *et al.* 1979). Dietary calcium restriction also increases active calcium transport which is paralleled by changes in calbindin-D9k in rats up to at least 12 months of age (Armbrecht *et al.* 1979). In contrast Armbrecht *et al.* (1984) found that rats over 12 months of age increased circulating 1,25-dihydroxyvitamin D, but did not increase calcium absorption in response to a low calcium diet. In the present experiment the rats were between 11·5 and 13·5 months of age and the difference in the two observations may be explained by different methods, with the present study using the *in vivo* calcium balance method, whereas the Armbrecht group determined calcium absorption *in vitro* using the everted gut sac technique.

Ovariectomy reduced calcium balance at all levels of dietary calcium due to increased faecal calcium excretion, a consequence of reduced intestinal calcium absorption. The intercept for the relationship between faecal calcium and dietary calcium consumption was 20% higher in the ovariectomized rats compared with sham rats, but ovariectomy did not affect the slope or the quadratic term for this relationship. This difference between the intercepts was more clearly demonstrated when only the data on rats fed diets containing from 0·02 to 0·2% Ca were included in the analysis, presumably because of a large coefficient of variation in calcium balance due to small differences between variables involving large amounts of calcium (Taagehøj Jensen *et al.* 1983).

With 99% of whole-body calcium stored in bone, calcium balance is analogous to bone balance. It has been well recognized that dietary calcium restriction decreases bone density in animals (Jowsey & Raisz, 1968) and more recently, Shen *et al.* (1995) demonstrated that bone loss occurring as a result of calcium deficiency and oestrogen deficiency are additive. From the equations describing the relationship between calcium consumption and calcium balance the minimum daily consumption of calcium required to maintain a neutral calcium balance on the AIN-76A-starch diet for sham rats is 0·45 mmol day^{-1} rising by tenfold for ovariectomized rats to 4·58 mmol day^{-1}. It is difficult to extrapolate these findings to other studies using diets of different composition, because components such as lactose (Favus & Angeid-Backman, 1984), starch (Schulz *et al.* 1993), protein content (Kerstetter & Allen, 1991), dietary fibre (Harmuth-Hoene & Schelenz, 1980) and other factors affecting calcium availability (Pansu *et al.* 1993) modulate the efficiency of intestinal calcium absorption. While 24 h urine calcium excretion was unaffected by ovariectomy, consistent with findings of other studies (Yamazaki & Yamaguchi, 1989; Morris *et al.* 1992), the relationship between urine calcium excretion and calcium consumption was also unaffected even though the efficiency of intestinal calcium absorption was impaired by ovariectomy. These data indicate that urine calcium excretion by ovariectomized rats was high relative to the amount of calcium absorbed from the diet. A small but significant rise in the fasting urine calcium excretion following ovariectomy in the rat has recently been reported (Morris *et al.* 1995). There was no effect of ovariectomy on intestinal calcium secretion in adult rats. In the young ovariectomized rat, we have previously identified an increase in intestinal calcium secretion detectable at 3 and 6 weeks post ovariectomy, but attenuated by 9 weeks post ovariectomy (O'Loughlin & Morris, 1994). Calcium balance was not measured in the present study until at least 10 weeks post ovariectomy, thus our data do not rule out the possibility of a transient effect of ovariectomy on intestinal calcium secretion in the adult rat within 10 weeks of operation as observed in the young rat.

The major factor leading to the more negative calcium balance in the ovariectomized rats was an impairment to net calcium absorption consistent with a previous report with rats fed a low dietary calcium (Kalu *et al.* 1989), although it was not detected at normal dietary calcium intakes. The present data suggest that calcium absorption is impaired regardless of the level of dietary calcium. Previous studies which have assessed calcium absorption in isolated intestinal

segments using isolated duodenal loops (Thomas & Ibarra, 1987; Thomas *et al.* 1988; Miller *et al.* 1991) and everted gut sacs (Lindgren & DeLuca, 1982) have not detected a decrease in calcium absorption following ovariectomy. Several of the isolated duodenal loop studies (Thomas & Ibarra, 1987; Thomas *et al.* 1988) actually reported an increase in active calcium transport. A possible explanation for the discrepancy between the balance studies and the isolated duodenal studies may be a difference in the contribution of passive absorption. Our data confirm that ovariectomy does not reduce circulating 1,25-dihydroxyvitamin D levels (Kalu *et al.* 1989). The impairment of true intestinal calcium absorption that follows ovariectomy is, therefore, not mediated by reduced production of 1,25-dihydroxyvitamin D. In fact after 12 weeks on the 0·02% Ca diet, the level of 1,25-dihydroxyvitamin D was significantly higher in the Ovx group compared with the sham group indicating stimulation of 1,25-dihydroxyvitamin D production. This finding is in sharp contrast with the notion of calcium malabsorption induced by reduced circulating 1,25-dihydroxyvitamin D that has been proposed for postmenopausal women (Gallagher *et al.* 1979). Therefore the impairment of intestinal calcium absorption as a result of ovariectomy despite elevated 1,25-dihydroxyvitamin D levels may have three possible explanations: (1) that there is an impaired intestinal response to 1,25-dihydroxyvitamin D, (2) 1,25-dihydroxyvitamin D active transport is saturated and cannot be further increased, or (3) the impairment to intestinal calcium absorption that follows ovariectomy is not an impairment to vitamin D-mediated calcium absorption. In the present study 1,25-dihydroxyvitamin D administration to rats fed a 0·05% Ca diet produced an equivalent increase in intestinal calcium absorption in both sham and ovariectomized groups, indicating no impairment of the 1,25-dihydroxyvitamin D response at the level of the gut. This finding is inconsistent with the proposal that ovariectomy leads to a downregulation of vitamin D receptors in the enterocyte (Chan *et al.* 1984; Chen & Kalu, 1995) or that the vitamin D-mediated intestinal calcium absorption is saturated following ovariectomy. Calcium balance was not improved with 1,25-dihydroxyvitamin D treatment of ovariectomized rats, because there were concomitant rises in the excretion of calcium at both the gut and the kidney. The reported rise in intestinal calcium absorption following 1,25-dihydroxyvitamin D treatment was increased in oestradiol-replaced ovariectomized women compared with placebo-treated ovariectomized women, suggesting that oestradiol influences vitamin D-mediated calcium absorption (Gennari *et al.* 1990). In postmenopausal osteoporotic women the relationship between 1,25-dihydroxyvitamin D and fractional calcium absorption had an equivalent slope but lower intercept than the relationship for normal postmenopausal women (Morris *et al.* 1991) indicating that the osteoporotic group had a constant negative bias for intestinal calcium absorption at all levels of circulating 1,25-dihydroxyvitamin D. An age-related decrease in intestinal responsiveness to 1,25-dihydroxyvitamin D due to a decrease in intestinal vitamin D receptors has been reported in normal women (Ebeling *et al.* 1992). Because there was also an age-related rise in 1,25-dihydroxyvitamin D there was no decrease in intestinal calcium absorption.

The administration of oestradiol to ovariectomized rats reversed the impairment in intestinal calcium absorption. Because the rise in intestinal calcium absorption was not brought about by a rise in circulating 1,25-dihydroxyvitamin D and the intestinal response to 1,25-dihydroxyvitamin D is unaffected by oestrogen deficiency, oestradiol appears to be acting directly on the gut to stimulate calcium absorption. Previous studies have indicated that there are significant changes to intestinal weight per unit length following ovariectomy (Miller *et al.* 1991), which may alter the characteristics of passive, paracellular calcium transport. Oestrogen receptors have been identified in a transformed rat enterocyte cell line (Thomas *et al.* 1993) and 17β-oestradiol has been shown to stimulate ^{45}Ca uptake in isolated rat enterocytes (Arjmandi *et al.* 1993) and *in vivo* intestinal calcium absorption in rats demonstrated by calcium balance studies (Arjmandi *et al.* 1994). More recently a negative calcium response element type 2 has been identified on the oestrogen receptor gene, consistent with the notion that oestrogens play a role in overall calcium homeostasis rather than just bone calcium balance (McHaffie & Ralston, 1995).

Unlike the findings with 1,25-dihydroxyvitamin D administration, oestradiol administration markedly improved calcium balance indicating that the extra calcium absorbed from the diet is mainly accreted to bone. This suggests that oestradiol also has a direct affect on bone cells shifting the balance between bone formation and bone resorption to achieve net accumulation of mineral. The elevated circulating PTH at this time suggests that serum ionized calcium was reduced concomitant with increased bone formation. Previous studies have indicated that oestradiol administration causes a rise in serum calcium (Kalu *et al.* 1989; Arjmandi *et al.* 1994), with no change in circulating PTH even though intestinal calcium absorption was stimulated (Arjmandi *et al.* 1994). However, the previous studies were performed on rats fed a calcium-sufficient diet (0·4%), whereas the present study used a diet low in calcium (0·05%). We have demonstrated that ovariectomy in the adult rat decreases calcium balance by decreasing intestinal calcium absorption; however, the magnitude of the adaptive response to dietary calcium restriction is unaffected by ovariectomy. Decreased intestinal calcium absorption in the ovariectomized rat is not the result of decreased circulating 1,25-dihydroxyvitamin D or a decreased intestinal response to 1,25-dihydroxyvitamin D, but was corrected by oestradiol replacement. Our findings suggest that oestradiol stimulates a non-vitamin D-mediated mechanism of intestinal calcium absorption.

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